# **WEST Search History**

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DATE: Tuesday, March 29, 2005

Hide?	Set Nam	<u>e Query</u>	Hit Count
	DB=PC	SPB,USPT; PLUR=YES; OP=ADJ	
	L7	15 and (plastid or chloroplast) [clm]	23
	L6	L4 and homologous recombination	321
	L5	L4 and (lox or cre or flp or frt)	177
	L4	L3 and transgenic	637
	L3	L2 and excis\$	848
	L2	L1 and site specific	1459
	L1	plastid or chloroplast	6569

END OF SEARCH HISTORY

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NEWS 1

Web Page URLs for STN Seminar Schedule - N. America

"Ask CAS" for self-help around the clock

NEWS 3

FEB 25

CA/CAPLUS - Russian Agency for Patents and Trademarks

(ROSPATENT) added to list of core patent offices covered

NEWS 4

FEB 28

PATDPAFULL - New display fields provide for legal status
data from INPADOC

NEWS 5

FEB 28

BABS - Current-awareness alerts (SDIs) available

NEWS 6

FEB 28

MEDLINE/LMEDLINE reloaded

NEWS 7

MAR 02

GBFULL: New full-text patent database on STN

NEWS 8

MAR 03

REGISTRY/ZREGISTRY - Sequence annotations enhanced

NEWS 9

MAR 03

MEDLINE file segment of TOXCENTER reloaded

NEWS 10

MAR 22

KOREAPAT now updated monthly; patent information enhanced

NEWS 11

MAR 22

Original IDE display format returns to REGISTRY/ZREGISTRY

NEWS 12

MAR 22

PATDPASPC - New patent database available

NEWS 13

MAR 22

REGISTRY/ZREGISTRY enhanced with experimental property tags

NEWS EXPRESS JANUARY 10 CURRENT WINDOWS VERSION IS V7.01a, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 10 JANUARY 2005

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FULL ESTIMATED COST

FILE 'AGRICOLA' ENTERED AT 17:11:00 ON 29 MAR 2005

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=> s plastid or chloroplast

1 80679 PLASTID OR CHLOROPLAST

=> s ll and site specific

L2 261 L1 AND SITE SPECIFIC

=> s 12 and transgenic

L3 47 L2 AND TRANSGENIC

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PROCESSING COMPLETED FOR L3

L4 34 DUP REM L3 (13 DUPLICATES REMOVED)

=> d 1-10 ti

- L4 ANSWER 1 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Unidirectional **site-specific** integration system for integrating a nucleic acid into the genome of a target cell
- L4 ANSWER 2 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Removal of heterologous sequences, such as selectable marker genes, from plastid genome by transiently expressed site-specific recombinases in higher plants
- L4 ANSWER 3 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Plant transformation with in vivo assembly of a sequence of interest
- L4 ANSWER 4 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Method of controlling cellular process in plants by externally applied signal
- L4 ANSWER 5 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN
- TI A novel approach to **plastid** transformation utilizes the phiC31 phage integrase.
- L4 ANSWER 6 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Selection of **transgenic** organisms by selecting for loss of a growth inhibiting marker gene
- L4 ANSWER 7 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Method for the transformation of vegetable plastids
- L4 ANSWER 8 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Stable transformation of plants by integration of transforming DNA into the **plastid** genome by homing nuclease-mediated homologous recombination
- L4 ANSWER 9 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI High level expression of immunogenic proteins in the plastids of higher plants and use thereof
- L4 ANSWER 10 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2005) on STN
- TI Identification of functional lox sites in the plastid genome.

=> d 2 so

L4 ANSWER 2 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN

SO PCT Int. Appl., 47 pp. CODEN: PIXXD2

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ANSWER 2 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
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    WO 2004078935
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            GN, GQ, GW, ML, MR, NE, SN, TD, TG, BF, BJ, CF, CG, CI, CM, GA,
            GN, GQ, GW, ML, MR, NE, SN, TD, TG
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## => d 2 ab

ANSWER 2 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
Compns. and methods for manipulating the plastid genome of higher plants are provided. The methods of the invention may be employed to remove predetd. target sequences from the plastid genome, such as selectable marker genes following successful isolation of transformed progeny. In one embodiment of the invention, the method entails providing a transplastomic plant cell comprising plastids having heterologous nucleic acid sequence(s) flanked by excision sites and a nucleic acid sequence encoding at least one foreign gene of interest which is not flanked by excision sites. The plant cell is then contacted with a DNA construct which comprises a promoter operably linked to a nucleic acid encoding a protein having excision activity such as CRE, resolvase, FLP, SSVI-encoded integrase, phiC31 integrase and transposases.

## => d 3 ab

ANSWER 3 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN L4A process of producing transgenic plants or plant cells stably AΒ transformed on a chromosome with a DNA sequence of interest and capable of expressing a function of interest from said DNA sequence of interest, said process comprising (a) providing plant cells or plants with at least two different vectors, whereby (i) said at least two different vectors are adapted to recombine with each other by site-specific recombination in said plant cells for producing a non-replicating recombination product containing said DNA sequence of interest, (ii) said at least two different vectors are adapted for integrating said DNA sequence of interest into said chromosome, (iii) said DNA sequence of interest contains sequence portions from at least two of said at least two different vectors, said sequence portions being necessary for expressing said function of interest from said DNA sequence of interest; and (b) selecting plants or plant cells expressing said function of interest.

# => d 2 pi

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IS, JP, JP, KE, KE, KG, KG, KP, KP, KP, KR, KR, KZ, KZ, KZ, LC, LK, LR, LS, LS, LT, LU, LV, MA, MD, MD, MG, MK, MN, MW, MX, MX, MZ, MZ, NA, NI

RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
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#### => d 6 ab

ANSWER 6 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN **T.4** The present invention relates to creation and selection of genetically AΒ modified organisms by site-specific integration and expression of toxin/antidote genes within a recombinant host genome. Homologous recombination enables targeted integration of the exogenous gene(s) within the genome of the cell or organism. A toxic gene from the gene set of CcdB, ParE, RelE, Kid, Doc, MazE, PemK and HoK, regulated by an inducible promoter, is integrated into a host cell line genome. An antidote gene from the gene set of CcdA, Kis, Phd, PemI, and Sok, also regulated by an inducible promoter, is integrated into the same recombinant cell line genome. Inducible promoters allow for tissue- or developmental stage-specific expression of the exogenous genes within the recombinant cell. Using the appropriate signal sequences, toxin and antidote protein expression can be targeted to the mitochondria or chloroplast. This expression system is used as a means for selection of genetically modified eukaryotic organisms, such as plants,

animals (mammals), and microbes (yeast).

# => d 6 pi

L4	ANSWER	6 OF 34	CAP	LUS	COPY	RIG	HT 2	005 .	ACS (	on S'	ΓN					
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ΡI	WO 2003	078638		A1	2	2003	0925	1	WO 2	003-1	BE45			20	0030	319
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	CA 2477	194		AA	2	2003	0925		CA 2	003-	2477	194		20	0030	319
	EP 1485	491		A1	2	004	1215		EP 2	003-	7094	57		20	0030	319
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## => d 7 ab

ANSWER 7 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN

Methods of achieving stable transformation of plants by integrating the transforming DNA into the plastid genome by sitespecific integration are described. Integration is achieved by the use of known sequence-specific recombination mechanisms, such as cre/loxP, or through the use of homing endonucleases with large recognition sequences. Plants are constructed in which a sequence-specific recombination site is introduced into the plastid DNA. These plants are then transformed with a DNA containing the cognate recombination sequence and the gene for the corresponding recombinase and transformants selected. The expression construct may include elements such as plastid-specific promoters and selection markers and the recombinase may be retained in the

plastid by a transit peptide. The method achieves a high
efficiency of integration of the transforming DNA into the plastid
DNA.

# => d 7 pi

L4	ANSWER	7 OF	34	CAP:	LUS	COP	YRIG.	HT 2	005 .	ACS (	on S'	TN					
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ΡĪ	WO 2003	05420	01		A1	:	2003	0703	1	WO 2	002-	EP14:	303		2	00212	216
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	EP 1461	CF, CC EP 1461439			A1	- :	2004	0929		EP 2	002-	8053	24		20	00212	216
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#### => d 8 ab

ANSWER 8 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN

Methods of achieving stable transformation of plants by integrating the transforming DNA into the plastid genome by sitespecific integration are described. Integration is achieved by the use of known sequence-specific recombination mechanisms, especially by the use of homing endonucleases with large recognition sequences. Plants are constructed in which a homing endonuclease cleavage site is introduced into the plastid DNA. These plants are then transformed with a DNA containing the cognate recombination sequence and the gene for the corresponding enzyme and transformants selected. The expression construct may include elements such as plastid-specific promoters and selection markers and the enzyme may be retained in the plastid by a transit peptide. The method achieves a high efficiency of integration of the transforming DNA into the plastid DNA.

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ANSWER 9 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN

As ite specific recombination system and methods of use thereof are disclosed for manipulating the genome of higher plants. The methods and systems of the invention may be employed to remove heterologous sequences from the plastid genome, such as selectable marker genes following successful isolation of transformed progeny. Alternatively, they may be designed to remove endogenous genes involved in plant cell metabolism, growth, development and fertility. Compns. and methods for expressing immunogenic proteins using the site specific recombination system are also provided.

## => d 9 pi

L4	ANSWER PATENT			COPYRIGHT 2 DATE	005 ACS on STN APPLICATION NO.	DATE
PI	US 2003 WO 2001		A1 A1		US 2002-109812 WO 2000-US25930	
	W :				BA, BB, BG, BR, BY, BZ	
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	WO 2003		A2		WO 2003-US9970	20030331
	WO 2003	083086	A3	20040819		
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	EP 1495		CF, CG,		EP 2003-718140	•
					GB, GR, IT, LI, LU, NL	
	ĸ.				CY, AL, TR, BG, CZ, EE	

#### => d 10 ab

- L4 ANSWER 10 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2005) on STN
- AB Our objective was to test whether or not cyclization recombination (CRE), the P1 phage site-specific recombinase, induces genome rearrangements in plastids. Testing was carried out in tobacco plants in which a DNA sequence, located between two inversely oriented locus of X-over of P1 (loxP) sites, underwent repeated cycles of inversions as a means of monitoring CRE activity. We report here that CRE mediates deletions between loxP sites and plastid DNA sequences in the 3' rps12 gene leader (lox-rps12) or in the psbA promoter core (lox-psbA). We also observed deletions between two directly oriented lox-psbA sites, but not between lox-rps12 sites. Deletion via duplicated rRNA operon promoter (Prrn) sequences was also frequent in CRE-active plants. However, CRE-mediated recombination is probably not directly involved, as no recombination junction between loxP and Prrn could be observed. Tobacco plants carrying deleted genomes as a minor fraction of the plastid genome population were fertile and phenotypically normal, suggesting that the absence of deleted genome segments was compensated by gene expression from wild-type copies. The deleted plastid genomes disappeared

in the seed progeny lacking CRE. Observed plastid genome rearrangements are specific to engineered plastid genomes, which contain at least one loxP site or duplicated psbA promoter sequences. The wild-type plastid genome is expected to be stable, even if CRE is present in the plastid.

=> d 10 pi

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- SO Plant journal, 2003 Sept. Vol. 35, no. 6 p. 753-762 ISSN: 0960-7412

=> d 11-20 ti

- L4 ANSWER 11 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 1
- TI Expression of the B subunit of E. coli heat-labile enterotoxin in the chloroplasts of plants and its characterization
- ANSWER 12 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 2
- TI Marker-free transgenic plants.
- L4 ANSWER 13 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 3
- TI Chloroplast Transformation in Oilseed Rape
- L4 ANSWER 14 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Antibiotic resistance genes in **transgenic** plants: their origins, undesirability and technologies for their elimination from genetically modified crops
- ANSWER 15 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2005) on STN
- TI The plastid clpP1 protease gene is essential for plant development.
- L4 ANSWER 16 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Methods of enhancing and optimizing expression of exogenes in transgenic plants
- L4 ANSWER 17 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Construction of bicistronic-transgene expression vectors containing internal ribosome entry site (IRES) regulated selectable marker for transgenic plants
- L4 ANSWER 18 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 4
- Analysis of chloroplast transformed tobacco plants with crylIa5 under rice psbA transcriptional elements reveal high level expression of Bt toxin without imposing yield penalty and stable inheritance of transplastome.

- L4 ANSWER 19 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN

  TI Site-specific integration of insect-resistant gene into chloroplast genome of oilseed rape and acquisition of transgenic plants
- L4 ANSWER 20 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 5
  TI Positive, negative and marker-free strategies for transgenic
  plant selection

=> d 11 ab

ANSWER 11 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 1 L4Transgenic chloroplasts have become attractive systems for AB heterologous gene expressions because of unique advantages. Here, we report a feasibility study for producing the nontoxic B subunit of Escherichia coli heat-labile enterotoxin (LTB) via chloroplast transformation of tobacco. Stable site-specific integration of the LTB gene into chloroplast genome was confirmed by PCR and genomic Southern blot anal. in transformed plants. Immunoblot anal. indicated that plant-derived LTB protein was oligomeric, and dissociated after boiling. Pentameric LTB mols. were the dominant mol. species in LTB isolated from transgenic tobacco leaf tissues. The amount of LTB protein detected in transplastomic tobacco leaf was approx. 2.5% of the total soluble plant protein, approx. 250-fold higher than in plants generated via nuclear transformation. The GM1-ELISA binding assay indicated that chloroplast-synthesized LTB protein bound to GM1-ganglioside receptors. LTB protein with biochem. properties identical to native LTB protein in the chloroplast of edible plants opens the way for inexpensive, safe, and effective plant-based edible vaccines for humans and animals.

=> d 22 so

L4 ANSWER 22 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN SO PCT Int. Appl., 26 pp.

CODEN: PIXXD2

=> d 22 pi

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ANSWER 22 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
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    PATENT NO.
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                               20010607
                                         WO 2000-US42086
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    WO 2001040492
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            LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,
            SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU,
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            BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
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    EP 1232275
                        A2
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                                          EP 2000-992497
        R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
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                               20040722 US 2004-755275
    US 2004143874
                        A1
                                                                20040113
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#### => d 11 so

- L4 ANSWER 11 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 1
- SO Transgenic Research (2003), 12(6), 683-691 CODEN: TRSEES; ISSN: 0962-8819

=> d 12 so

- ANSWER 12 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 2
- SO Plant cell, tissue and organ culture, 2003 Aug. Vol. 74, no. 2 p. 123-134 ISSN: 0167-6857
- => d 12 ab
- L4 ANSWER 12 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 2
- AB Selectable marker genes are widely used for the efficient transformation of crop plants. In most cases, selection is based on antibiotic or herbicide resistance. Due mainly to consumer concerns, a suite of strategies (site-specific recombination, homologous recombination, transposition and co-transformation) have been developed to eliminate the marker gene from the nuclear or chloroplast genome after selection. Current efforts concentrate on systems where marker genes are eliminated efficiently soon after transformation. Alternatively, transgenic plants are produced by the use of marker genes that do not rely on antibiotic or herbicide resistance but instead promote regeneration after transformation. Here, the merits and shortcomings of different approaches and possible directions for their future development are discussed.

# => d 13 b

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In a multifile environment, a format can only be used if it is valid in at least one of the files. Refer to file specific help messages or the STNGUIDE file for information on formats available in individual files.

REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):ab

T.4 ANSWER 13 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 3 The chloroplast transformation vector pNRAB carries two AB expression cassettes for the spectinomycin resistance gene aadA and the insect resistance gene cry1Aa10. The two cassettes are sited between the rps7 and ndhB targeting fragments. Biolistic delivery of the vector DNA, followed by spectinomycin selection, yielded chloroplast transformants at a frequency of four in 1000 bombarded cotyledon petioles. PCR anal. and Southern blot of PCR products confirmed the sitespecific integration of aadA and crylAalO into the chloroplast genomes of transgenic oilseed rape. When transgenic oilseed rape leaves were fed to second instar Plutella xylostera larvae, 47% mortality was observed against this insect and the surviving larvae had significantly lower weight than the control. This is the first report of chloroplast transformation in oilseed rape and the introduction of novel genes between the rps7 and ndhB genes in the chloroplast genome. This offers an opportunity for improvement of oilseed rape by chloroplast genetic engineering.

L4 ANSWER 13 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 3 SO Transgenic Research (2003), 12(1), 111-114

CODEN: TRSEES; ISSN: 0962-8819

#### => d 14 ab

ANSWER 14 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN T.4 A review, with refs. Plant transformation technologies use antibiotic AB resistance genes as markers to identify the small fraction of transgenic cells that have taken up trait genes. In addition to plant selectable marker genes, vector-localized genes such as the ampicillin resistance bla(TEM1) gene, can also integrate into the chromosomes of transgenic plants. Integration of vector sequences is particularly problematic when whole plasmids integrate into plant nuclear DNA following their transfer into cells by artificial DNA delivery methods such as particle bombardment. Microbial resistance to antibiotics threatens the success of infectious disease treatment and prevention in the 21st century. While the risk of horizontal transfer of antibiotic resistance genes is minuscule, their elimination from genetically manipulated crops provides a simple solution for ending the continuing debate over the likelihood of pathogen acquisition of plant-derived antibiotic resistance genes. To avoid the presence of antibiotic resistance genes in transgenic crops, they can be removed once they have served their purpose or they can be replaced with alternative marker genes. These two approaches are not mutually exclusive and can be combined where needed to avoid safety evaluations on each new marker gene. This chapter reviews technologies for removing antibiotic resistance genes from transgenic plants and describes an expanding list of alternative marker genes that do not require antibiotic selection. Plastid engineering illustrates the ease with which both antibiotic resistance genes and vector sequences can be removed from plants using homologous recombination. Efficient marker gene excision technologies and alternative marker genes combine for a better toolkit for the next generation of transgenic crops. This toolkit will facilitate multiple rounds of transformation with the best marker for a particular crop and allow the removal of all excess foreign DNA from a crop. As a consequence the focus of attention will shift from the marker genes to the all important trait genes that are responsible for the added value of genetically manipulated crops.

## => d 14 so

- L4 ANSWER 14 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- SO Transgenic Plants (2003), 111-156. Editor(s): Stewart, C. Neal, Jr. Publisher: Horizon Scientific Press, Wymondham, UK.

CODEN: 69ECK5; ISBN: 1-898486-44-1

#### => d 15 ab

- L4 ANSWER 15 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2005) on STN
- AB Plastids of higher plants are semi-autonomous cellular organelles that have their own genome and transcription-translation machinery. Examples of plastid functions are photosynthesis and biosynthesis of starch, amino acids, lipids and pigments. Plastid functions are encoded in 120 plastid genes and 3,000 nuclear genes. Although many embryo and seedling lethal nuclear genes are required for chloroplast biogenesis, until now deletion of plastid genes either had no phenotypic consequence (8 genes), or caused a mutant phenotype but did not affect viability (13 genes). Here we identify an essential plastid gene. By using the CRE-lox site-specific recombination system we have deleted clpP1 (caseinolytic protease P1), one of the three genes (clpP1, ycf1 and ycf2) whose

disruption had previously only been possible in a fraction of the 1,000-10,000 plastid genome copies in a cell. Loss of the clpP1 gene product, the ClpP1 protease subunit, results in ablation of the shoot system of tobacco plants, suggesting that ClpP1-mediated protein degradation is essential for shoot development.

## => d 15 so

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  (2005) on STN
- SO Nature, 2003 Sept. 4 Vol. 425, no. 6953 p. 86-89 ISSN: 0028-0836

## => d 20 so

- L4 ANSWER 20 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 5
- SO Brazilian Journal of Plant Physiology (2002), 14(1), 1-10 CODEN: BJPPBR; ISSN: 1677-0420

## => d 21-30 ti

- L4 ANSWER 21 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Excision of selection marker gene in **transgenic** plant for reducing health and environment risk
- L4 ANSWER 22 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Inducible **site-specific** recombination for the activation and removal of transgenes in **transgenic** plants
- L4 ANSWER 23 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Site-specific recombination system to manipulate the plastid genome of higher plants
- L4 ANSWER 24 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Removal of antibiotic resistance genes from **transgenic** tobacco plastids. [Erratum to document cited in CA135:14859]
- ANSWER 25 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 6
- TI Efficient elimination of selectable marker genes from the plastid genome by the CRE-lox site-specific recombination system.
- L4 ANSWER 26 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 7
- TI Edited transcripts compete with unedited mRNAs for trans-acting editing factors in higher plant chloroplasts
- L4 ANSWER 27 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Removal of antibiotic resistance genes from transgenic tobacco plastids
- L4 ANSWER 28 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Green fluorescent protein expression constructs for use as a screenable marker for plant transformation
- L4 ANSWER 29 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 8
- TI A heterologous maize rpoB editing site is recognized by transgenic tobacco chloroplasts
- L4 ANSWER 30 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States

of America. It contains copyrighted materials. All rights reserved. (2005) on STN

TI In vivo dissection of cis-acting determinants for **plastid** RNA editing.

## => d 21 ab

ANSWER 21 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN L4 AB Antibiotic resistance gene is commonly used as selection marker in construction of transgenic plants. However, the inheritance of antibiotic resistance gene is thought as a risk for health and environment. The invention provides method for producing a transgenic plant comprising a recombinant plastid genome containing an exogenous gene in the absence of a selectable marker gene introduced with the exogenous gene by using direct repeat sequences, nucleic acid constructs containing direct repeat sequences which may be used in the method and transgenic plants produced by the method. invention provides detailed description about plasmid construction, transformation, excision of marker gene by irrigation and crossing. transgenic tobacco provides in this invention without selection marker bar gene showed resistance to herbicide. The method provides in this invention can be used to improve the quality of transgenic crop plants by producing genetic hazard free plants.

#### => d 21 so

L4 ANSWER 21 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN SO PCT Int. Appl., 59 pp. CODEN: PIXXD2

# => d 21 pi

L4	ANSWER PATENT			KIN	D DA	ATE		1	APPL	CAT:	I NO			D	ATE	
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#### => d 22 ab

ANSWER 22 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN

Disclosed is an inducible promoter system in conjunction with a 
site-specific recombination system which allows (i)

specific activation of transgenes at specific times or (ii) excision and removal of transgenes (e.g., antibiotic resistance markers) from 
transgenic plants. These "suicide" gene cassettes, including the 
recombination system itself, can be evicted from the plant genome once 
their function has been exerted. The system is based on the ability to 
temporally and spatially induce the expression of CRE recombinase which

then binds to directly repeated lox sites flanking the transgene in question leading to the precise excision of the gene cassette. Also disclosed is a method to activate an inverted, and therefore silent, transgene by placing two lox sites in opposite orientations flanking the transgene. This results in inversion of the intervening DNA fragment in the presence of CRE recombinase. This activation can be timed by placing the CRE recombinase under the control of an inducible promoter. In order to test this system a construct was designed that allows in planta monitoring of precise excision events using the firefly luciferase (LUC) reporter gene as a marker for recombination.

## => d 22 pi

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	US	2004											7552	75		2	0040	113

## => d 23 ab

ANSWER 23 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN L4AB A site specific recombination system and methods of use thereof are disclosed for manipulating the genome of higher plants. The present invention provides a bacteriophage P1 CRE-loxP sitespecific recombination system which is suitable for efficient elimination of marker genes from the plastid genome. The system exemplified has two components : a plastid tester strain carrying a cytosine deaminase (codA) transgene flanked by lox sites conferring sensitivity to 5-fluorocytosine and a nuclear CRE line carrying a nuclear-encoded, plastid-targeted CRE. The selection marker gene codA is eliminated at a very fast rate when the plastid -targeted CRE is introduced into the plastid tester strain by transformation or crossing. CRE-mediated inversion reaction can be achieved by inverting the orientation of the marker gene (flanked by inverted lox sites) relative to its promoter. The method can be used to obtain marker free transplastomic plants through Cre-mediated deletion, for high level expression of recombinant proteins and to obtain cytoplasmic male sterility by deleting vital plastid genes.

## => d 23 pi

L4	ANSWER PATENT		F 34		PLUS KIN		PYRI DATE						NO.		Di	ATE	
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ΡI	WO 2001	0217	68		A1		2001	0329	1	WO 2	000-1	JS25	930		2	0000	921
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US 2003088081
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                             20030508
                                         US 2002-109812
                                                                   20020329
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## => d 24 ab

L4 ANSWER 24 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN

AB In the Exptl. Protocol under "Generation and anal. of transplastomic plants", the values of several compds. were incorrectly noted. Shoots and green cell lines were selected on spectinomycin dihydrochloride pentahydrate (Duchefa, Haarlem, The Netherlands) plus streptomycin sulfate (Sigma, St. Louis, MO), each at 500  $\mu$ g/mL. Clones were transferred to RMOP medium containing 5  $\mu$ /mL glufosinate-ammonium (Dr. Ehrenstorfer GmbH, Augsburg, Germany) after 9-34 wk for a second cycle of regeneration. The 42 herbicide-resistant clones were rooted on MS medium containing 1  $\mu$ /mL glufosinate-ammonium and transferred to soil.

#### => d 24 so

L4 ANSWER 24 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN

SO Nature Biotechnology (2001), 19(2), 173

CODEN: NABIF9; ISSN: 1087-0156

#### => d 25 ab

- ANSWER 25 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 6
- AB Incorporation of a selectable marker gene during transformation is essential to obtain transformed plastids. However, once transformation is accomplished, having the marker gene becomes undesirable. Here we report on adapting the P1 bacteriophage CRE-lox site-specific recombination system for the elimination of marker genes from the plastid genome. The system was tested by the elimination of a negative selectable marker, codA, which is flanked by two directly oriented lox sites (>codA>). Highly efficient elimination of >codA> was triggered by introduction of a nuclear-encoded plastid-targeted CRE by Agrobacterium transformation or via pollen. Excision of >codA> in tissue culture cells was frequently accompanied by a large deletion of a plastid genome segment which includes the tRNA-Val(UAC) gene. However, the large deletions were absent when cre was introduced by pollination. Thus pollination is our preferred protocol for the introduction of cre. Removal of the >codA> coding region occurred at a dramatic speed, in striking contrast to the slow and gradual build-up of transgenic copies during plastid transformation. The nuclear cre gene could subsequently be removed by segregation in the seed progeny. The modified CRE-lox system described here will be a highly efficient tool to obtain marker-free transplastomic plants.

#### => d 25 so

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(2005) on STN

DUPLICATE 6

SO The Plant journal: for cell and molecular biology, July 2001. Vol. 27,

No. 2. p. 171-178

Publisher: Oxford: Blackwell Sciences Ltd.

ISSN: 0960-7412

=> d 25 au

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(2005) on STN

DUPLICATE 6

AU Corneille, S.; Lutz, K.; Svab, Z.; Maliga, P.

=> d 26 ab

ANSWER 26 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 7 Chloroplast RNA transcripts of vascular plants undergo C to U AB editing at approx. 30 sites, but there is no consensus sequence that identifies a C to be edited. Both sequences closely surrounding an edited C and unidentified **site-specific** trans-acting factors have been shown to be important for editing. The ability of an already edited transgenic sequence to bind and thus titrate a trans-acting editing factor was evaluated for two editing sites, ndhF and rpoB site 2. The U-containing rpoB transcripts did not affect editing of the endogenous rpoB transcripts, likely because the comparable C-containing transcripts containing 27 nucleotides surrounding the edited C were only 20% edited, indicating a low affinity of a trans-factor for this length of edited sequence. Surprisingly, U-containing ndhF transgene transcripts reduced endogenous ndhF transcript editing to the same degree as a C-containing transgene transcript. This indicates that the C target of editing is not a critical recognition feature for the sitespecific trans-acting factor.

=> d 27 sab

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In a multifile environment, a format can only be used if it is valid in at least one of the files. Refer to file specific help messages or the STNGUIDE file for information on formats available in individual files.

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ANSWER 27 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN Removal of antibiotic resistance genes from genetically modified (GM) AΒ crops removes the risk of their transfer to the environment or gut microbes. Integration of foreign genes into plastid DNA enhances containment in crops that inherit their plastids maternally. Efficient plastid transformation requires the aadA marker gene, which confers resistance to the antibiotics spectinomycin and streptomycin. The authors have exploited plastid DNA recombination and cytoplasmic sorting to remove aadA from transplastomic tobacco plants. A 4.9 kbp insert, composed of aadA flanked by bar and uidA genes, was integrated into plastid DNA and selected to remove wild-type plastid genomes. The bar gene confers tolerance to the herbicide glufosinate despite being GC-rich. Excision of aadA and uidA mediated by two 174 bp direct repeats generated aadA-free T0 transplastomic plants containing the bar gene. Removal of aadA and bar by three 418 bp direct repeats allowed the isolation of marker-free T2 plants containing a plastid-located uidA reporter gene.

=> d 27 so

L4 ANSWER 27 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN SO Nature Biotechnology (2000), 18(11), 1172-1176

CODEN: NABIF9; ISSN: 1087-0156

L4 ANSWER 27 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN AU Lamtham, Siriluck; Day, Anil

#### => d 28 ab

T.4

AB

ANSWER 28 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN A method for the production of transgenic plants is provided in which a vector carrying a gene encoding the green fluorescent protein is introduced into cells, the cells are screened for the protein and transformed cells are selected and regenerated. The cellular toxicity of the green fluorescent protein is circumvented by regulating expression of the gene encoding the protein or directing the protein to a subcellular compartment where it is not toxic to the cell. DNA constructs are provided for cell transformation in which the expression of a gene encoding the green fluorescent protein is placed under the control of an inducible promoter. In addition, DNA constructs are provided in which a nucleotide sequence encoding the green fluorescent protein is operably linked to a signal sequence which directs the expressed protein to a subcellular compartment where the protein is not toxic to the cell. Oxidative stress to plant cells transformed with GFP also can be ameliorated by transforming cells with an expression vector comprising genes encoding GFP and an oxygen scavenger enzyme such as superoxide dismutase. The toxicity of GFP in transformed plants can be eliminated by excising the screenable marker gene following detection of transformed cells or sectors. The FLP/FRT system is used in conjunction with GFP as a visible marker for transformation and FRT excision. A nucleotide sequence optimized for expression of the green fluorescent protein in plants is also provided. The use of the protein as a marker in the transformation and regeneration of maize is described. The efficiency of transformation with the GFP screenable marker was comparable to that with bialaphos as selectable marker.

# => d 28 pi

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<sup>=&</sup>gt; d 29 ab

L4 ANSWER 29 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 8

AB Single nucleotides in plant **chloroplast** transcripts are edited from the genomically encoded C to U, often resulting in changes of the

encoded protein sequence. Site-specific trans-acting factors are postulated to direct the selection of edited residues. order to further define cis sequences required for RNA editing, we investigated whether two editing sites present in maize  $\operatorname{rpoB}$  mRNA would be recognized by the editing machinery of transformed tobacco chloroplasts. A 93-nucleotide (nt) segment surrounding site I is sufficient to direct editing of the maize sequence in tobacco chloroplasts. However, an 86-nt segment surrounding maize site IV (which is genomically encoded as a T in tobacco) does not confer editing of this site, suggesting that trans-acting factors necessary for recognition of site IV are not present in tobacco. The maize sequences surrounding site I were found to compete with the endogenous rpoB for a depletable trans factor and to reduce editing of endogenous site I. The presence of exogenous maize site I was also found to decrease editing of endogenous tobacco site II, indicating that there is a shared aspect of editing for some closely spaced editing sites.

## => d 30 ab

- L4 ANSWER 30 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2005) on STN
- Substitutional RNA editing changes single C nucleotides in higher plant AB chloroplast transcripts into U residues. To determine the cis-acting sequence elements involved in plastic RNA editing, we constructed a series of chloroplast transformation vectors harboring selected editing sites of the tobacco ndhB transcript in a chimeric context. The constructs were inserted into the tobacco plastic genome by biolistic transformation leading to the production of stable chimeric RNAs. Analysis of RNA editing revealed unexpected differences in the size of the essential cis elements or in their distance from the editing site. Flanking sequences of identical size direct virtually complete editing for one pair of editing sites, partial editing for a second and no editing at all for a third pair of sites. Serial 5' and 3' deletions allowed us to define the cis-acting elements more precisely and to identify a sequence element essential for editing site recognition. In addition, a single nucleotide substitution immediately upstream of an editing position was introduced. This mutation was found drastically and selectively to reduce the editing efficiency of the downstream editing site, demonstrating that position -1 is important for either site recognition or catalysis. Our results indicate that the editing of adjacent sites is likely to be mechanistically coupled. In no case did the presence in the plastome of the additional editing sites have any effect on the editing efficiency of the endogenous ndhB sites, indicating that the availability of **site-specific** trans-acting factors is not rate limiting.

## => d 31-34 ti

- L4 ANSWER 31 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2005) on STN
- TI Site-specific factor involved in the editing of the psbL mRNA in tobacco plastids.
- ANSWER 32 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2005) on STN
- TI Introduction of a heterologous editing site into the tobacco **plastid** genome: the lack of RNA editing leads to a mutant phenotype.
- L4 ANSWER 33 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN

- TI Evidence for T-DNA mediated gene targeting to tobacco chloroplasts
- ANSWER 34 OF 34 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN

  DUPLICATE 9
- TI GT-1 binding site confers light responsive expression in transgenic tobacco.

=> dis his

L1

T<sub>1</sub>2

L3

T.4

(FILE 'HOME' ENTERED AT 17:09:18 ON 29 MAR 2005)

34 DUP REM L3 (13 DUPLICATES REMOVED)

FILE 'AGRICOLA, CAPLUS, BIOSIS' ENTERED AT 17:11:00 ON 29 MAR 2005 80679 S PLASTID OR CHLOROPLAST 261 S L1 AND SITE SPECIFIC 47 S L2 AND TRANSGENIC

=> s l1 and recombinase

=> s 11 and recombinase L5 20 L1 AND RECOMBINASE

=> dup rem ml5
ENTER L# LIST OR (END):l5
'ML5' IS NOT VALID. VALID FILE NAMES ARE 'AGRICOLA, CAPLUS, BIOSIS'
You have entered a file name of duplicates to keep that is not referenced by any of the L#s specified for this DUPLICATE command. The file names of duplicates that can be kept are listed above. Please enter one of these file names.

=> dup rem 15
PROCESSING COMPLETED FOR L5
L6 14 DUP REM L5 (6 DUPLICATES REMOVED)

=> d 1-10 ti

- L6 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Removal of heterologous sequences, such as selectable marker genes, from plastid genome by transiently expressed site-specific recombinases in higher plants
- L6 ANSWER 2 OF 14 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 1
- TI A novel approach to **plastid** transformation utilizes the phiC31 phage integrase.
- L6 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2
- TI Generation of marker-free **plastid** transformants using a transiently cointegrated selection gene
- L6 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Method for enhancing plant **plastid** transformation efficiency using procaryotic **recombinase** gene recA
- L6 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Method for the transformation of vegetable plastids
- L6 ANSWER 6 OF 14 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 3
- TI Identification of functional lox sites in the plastid genome.
- L6 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Double D-loop formation in duplex nucleic acid with recombinase

and modified oligonucleotides and applications

- L6 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Use of integrases to promote the insertion of foreign DNA into the plastid genome
- L6 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Construction of bicistronic-transgene expression vectors containing internal ribosome entry site (IRES) regulated selectable marker for transgenic plants
- L6 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Inducible site-specific recombination for the activation and removal of transgenes in transgenic plants
- => d 3 ab
- L6 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2 Genetic engineering of higher plant plastids typically involves stable AB introduction of antibiotic resistance genes as selection markers. though chloroplast genes are maternally inherited in most crops, the possibility of marker transfer to wild relatives or microorganisms cannot be completely excluded. Furthermore, marker expression can be a substantial metabolic drain. Therefore, efficient methods for complete marker removal from plastid transformants are necessary. One method to remove the selection gene from higher plant plastids is based on loop-out recombination, a process difficult to control because selection of homoplastomic transformants is unpredictable. Another method uses the CRE/lox system, but requires addnl. retransformation and sexual crossing for introduction and subsequent removal of the CRE recombinase. Here we describe the generation of marker-free chloroplast transformants in tobacco using the reconstitution of wild-type pigmentation in combination with plastid transformation vectors, which prevent stable integration of the kanamycin selection marker. benefit of a procedure using mutants is that marker-free plastid transformants can be produced directly in the first generation (TO) without retransformation or crossing.
- => d 3 pi
- L6 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2
- => d 3 so
- L6 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2 SO Nature Biotechnology (2004), 22(2), 225-229 CODEN: NABIF9; ISSN: 1087-0156
- => d 4 ab
- ANSWER 4 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN

  The objective of this invention is to enhance the efficiency of plastid transformation using nuclear transformed plants in which the microbial recombinase A(recA) is to target to (or expressed in) the plastid. This invention will be better explained by the following detailed descriptions. A plant is transformed with a nuclear transformation vector containing the microbial recA gene added with a plastid targeting sequence. In this nuclear transformed plant, the frequency of plastid transformation is enhanced greater than two-folds due to increased homologous recombination between the plastid transformation vector carrying genes of interest (or target genes) and the plastid genome. In addition, because plastid transformation is accomplished through a gradual process, adventitious shoots selected after being subjected to plastid

transformation should be cut into explants, and then shoots regenerated from the explants are to be reselected until all of the plastids in the shoots are uniformly transformed. However, when the nuclear transformed plant is used, the number of reselection is reduced to 1/2 to 1/3 due to increased homologous recombination.

## => d 4 pi

L6	ANSWER	4 OF 14	L CAP	LUS	COP	YRIG	HT 2	005 .	ACS (	on S'	TN					
	PATENT I	NO.		KIN	D :	DATE			APPL	ICAT	ION	NO.		D	ATE	
					-									-		
ΡI	WO 2003	060137		A1		2003	0724	1	WO 2	002-	KR25	06		2	0021	231
	W:	AE, AG	3, AL,	AM,	AT,	AU,	AZ,	BA,	BB,	BG,	BR,	BY,	BZ,	CA,	CH,	CN,
		CO, CI	R, CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	EŚ,	FI,	GB,	GD,	GE,	GH,
		GM, HI	R, HU,	ID,	IL,	IN,	IS,	JP,	KE,	KG,	KP,	KZ,	LC,	LK,	LR,	LS,
		LT, L	J, LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,	MZ,	NO,	NZ,	OM,	PH,	PL,
		, RU,	SC,	SD,	SE,	SG,	SK,	SL,	TJ,	TM,	TN,	TR,	TT,	TZ,	UA,	
		UG, US	S, UZ,	VC',	VN,	YU,	ZA,	ZM,	zw							
	RW:	GH, GI	1, KE,	LS,	MW,	MZ,	SD,	SL,	SZ,	TZ,	UG,	ZM,	ZW,	AM,	AZ,	BY,
		KG, K	Z, MD,	RU,	TJ,	TM,	AT,	BE,	BG,	CH,	CY,	CZ,	DE,	DK,	ĒE,	ES,
		FI, F	R, GB,	GR,	ΙE,	IT,	LU,	MC,	NL,	PT,	SE,	SI,	SK,	TR,	BF,	ВJ,
		CF, C	3, CI,	CM,	GA,	GN,	GQ,	GW,	ML,	MR,	ΝE,	SN,	TD,	TG		
	KR 2002	027383		Α	•	2002	0413		KR 2	002-	218			2	0020	103

#### => d 8 ab

ANSWER 8 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN

Compns. and methods for improving the efficiency of plastid transformation of plants are described. The method involves using a recombinase to promote integration of the transforming DNA into the plastid. The method has several stages. In the first stage a recombination site is introduced into the plastid DNA. The transformed line is the transformed with a vector including the gene of interest, and a selectable marker if necessary, and a transient expression cassette for an integrase or recombinase recognizing the recombination site. This promotes integration of the transforming DNA into the plastid DNA. Excision of the insert is prevented by limiting expression of the recombinase gene. Alternatively, the integrase gene may also be stably integrated into the plastid genome.

# => d 8 pi

L6	ANSWER PATENT												NO.		Dž	ATE	
PI	WO 2002 WO 2002									WO 2	002-1	US95	37		2	0020	329
	W:	AE, CO, GM, LS, PL, UA, GH, KG,	AG, CR, HR, LT, PT, UG, GM, KZ, IE,	AL, CU, HU, LU, RO, US, KE, MD, IT,	AM, CZ, ID, LV, RU, UZ, LS, RU, LU,	AT, DE, IL, MA, SD, VN, MW, TJ, MC,	AU, DK, IN, MD, SE, YU, MZ, TM, NL,	AZ, DM, IS, MG, SG, ZA, SD, AT, PT,	DZ, JP, MK, SI, ZM, SL, BE, SE,	SZ, CH, TR,	EE, KG, MW, SL, TZ, CY,	ES, KP, MX, TJ, UG, DE,	FI, KR, MZ, TM, ZM, DK,	GB, KZ, NO, TN, ZW, ES,	GD, LC, NZ, TR, AM, FI,	GE, LK, OM, TT, AZ, FR,	GH, LR, PH, TZ, BY, GB,
	US 2004	•	~ .	•	•	•	NE, 2004	•	•		004-	4732	07		20	0040	310

# => d 10 ab

AΒ

- L6 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
  - Disclosed is an inducible promoter system in conjunction with a

site-specific recombination system which allows (i) specific activation of transgenes at specific times or (ii) excision and removal of transgenes (e.g., antibiotic resistance markers) from transgenic plants. These "suicide" gene cassettes, including the recombination system itself, can be evicted from the plant genome once their function has been exerted. The system is based on the ability to temporally and spatially induce the expression of CRE recombinase which then binds to directly repeated lox sites flanking the transgene in question leading to the precise excision of the gene cassette. Also disclosed is a method to activate an inverted, and therefore silent, transgene by placing two lox sites in opposite orientations flanking the transgene. This results in inversion of the intervening DNA fragment in the presence of CRE recombinase. This activation can be timed by placing the CRE recombinase under the control of an inducible promoter. In order to test this system a construct was designed that allows in planta monitoring of precise excision events using the firefly luciferase (LUC) reporter gene as a marker for recombination.

# => d 10 pi

L6	ANSWER	10 OF 1	4 CAPLU	S COPYRIGHT	2005 ACS on STN					
	PATENT	NO.	KI	ND DATE	APPLICATION NO.	DATE				
ΡI	WO 2003	1040492	A	2 2001060	7 WO 2000-US42086	20001113				
	WO 2003	1040492	A	3 2002020						
	W :	AE, AG	, AL, AM	, AT, AU, AZ	, BA, BB, BG, BR, BY,	BZ, CA, CH, CN,				
		CR, CU	, CZ, DE	, DK, DM, DZ	, EE, ES, FI, GB, GD,	GE, GH, GM, HR,				
		HU, ID	, IL, IN	, IS, JP, KE	, KG, KP, KR, KZ, LC,	LK, LR, LS, LT,				
		LU, LV	, MA, MD	, MG, MK, MN	, MW, MX, MZ, NO, NZ,	PL, PT, RO, RU,				
		SD, SE	, SG, SI	, SK, SL, TJ	, TM, TR, TT, TZ, UA,	UG, UZ, VN, YU,				
		ZA, ZW	, AM, AZ	, BY, KG, KZ	, MD, RU, TJ, TM					
	RW:	GH, GM	, KE, LS	, MW, MZ, SE	, SL, SZ, TZ, UG, ZW,	AT, BE, CH, CY,				
		DE, DK	, ES, FI	, FR, GB, GR	, IE, IT, LU, MC, NL,	PT, SE, TR, BF,				
		BJ, CF	, CG, CI	, CM, GA, GN	, GW, ML, MR, NE, SN,	TD, TG				
	US 6723	3896	В	1 2004042	0 US 1999-439534	19991112				
	CA 2393	1312	Α	A 2001060	20001113					
	EP 1232	2275	A	2 2002082	1 EP 2000-992497	20001113				
	R:	AT, BE	, CH, DE	, DK, ES, FR	, GB, GR, IT, LI, LU,	NL, SE, MC, PT,				
		IE, SI	, LT, LV	, FI, RO, MK	, CY, AL, TR					
	US 2004	1143874	A	1 2004072	2 US 2004-755275	20040113				

#### => d 11-14 ti

- L6 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Site-specific recombination in plant cell plastids via transit peptiderecombinase fusion expression
- L6 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Site-specific recombination system to manipulate the **plastid** genome of higher plants
- L6 ANSWER 13 OF 14 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
- TI Efficient elimination of selectable marker genes from the plastid genome by the CRE-lox site-specific recombination system.
- ANSWER 14 OF 14 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN

  DUPLICATE 4
- TI The chloroplast-located homolog of bacterial DNA recombinase.

L6 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN AB By this invention, constructs and methods for genetic engineering of plant cells to provide for site-specific recombination of foreign DNA sequences inserted into the plant plastid are provided. In particular, plastid constructs are provided that comprise at least one DNA sequence, and at least two recombining sites. Particularly preferred constructs are those that employ Lox recombining sites. Another aspect of the present invention are recombinant nucleic acid constructs comprising a transcription initiation region functional in a plant cell, an organelle targeting sequence, and a nucleic acid sequence encoding recombinase. Also considered part of the present invention are the plants and plant cells comprising the constructs of the present invention. Another aspect of the present invention is to provide methods for directing site-specific recombination in a host plant cell

# => d 11 pi

plastid.

L6 2	ANSWER	11 OF	L4 CA	PLUS	COPY	YRIGHT	2005	ACS	on	STN							
1	PATENT NO.			KIND DATE				APPLICATION NO.					DATE				
							•										
PI V	WO 2001029241			A2 20010426 WO 2000-US28620					620	20001016							
1	WO 2001		A3 20011129														
	W:	AE, A	J, AM,	AT,	AU, A	AZ, BA,	BB,	BG,	BR,	BY,	CA,	CH,	CN,	CR,	CU,		
		CZ, D	E, DK,	DM,	EE, E	ES, FI,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,	IL,		
		IN, I	S, JP,	ΚE,	KG, F	KP, KR,	KZ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,		
		MD, MO	3, MK,	MN,	MW, N	NO, XI	NZ,	PL,	PT,	RO,	RU,	SD,	SE,	SG,	SI,		
		SK, S	J, TJ,	TM,	TR, T	TT, TZ,	UA,	ŪĠ,	UZ,	VN,	YU,	ZA,	ZW,	AM,	AZ,		
		BY, K	3, KZ,	MD,	RU, 1	rj, TM											
	RW:	GH, GI	1, KE,	LS,	MW, N	MZ, SD,	SL,	SZ,	TZ,	UG,	ZW,	ΑT,	ΒĒ,	CH,	CY,		
		DE, D	K, ES,	FI,	FR, C	GB, GR,	ΙE,	IT,	LU,	MC,	NL,	PT,	SE,	BF,	ВJ,		
		CF, C	G, CI,	CM,	GA, C	GN, GW,	ML,	MR,	ΝE,	SN,	TD,	TG					
(	CA 2387876			AA	AA 20010426			CA 2000-2387876					20001016				
]	BR 2000014858			Α	20	BR 2000-14858					20001016						
1	EP 1222296						,	EP 2000-972211									
	R:	AT, B	E, CH,	DE,	DK, E	ES, FR,	GB,	GR,	IT,	LI,	LU,	NL,	SE,	MC,	PT,		
		IE, S	, LT,	LV,	FI, F	RO, MK,	CY,	AL		-	_	-			,		
τ								US 2000-688851					20001016				

## => d 12 pi

L6	ANSWER	12 OF 14	CAPLUS	COPYRIGHT	2005 ACS on STN				
	PATENT NO.			D DATE	APPLICATION NO.	DATE			
ΡI	WO 2001	021768	A1	20010329	20000921				
	<b>W</b> :	AE, AG,	AL, AM,	AT, AU, AZ,	BA, BB, BG, BR, BY,	BZ, CA, CH, CN,			
		CR, CU,	CZ, DE,	DK, DM, DZ,	EE, ES, FI, GB, GD,	GE, GH, GM, HR,			
		HU, ID,	IL, IN,	IS, JP, KE,	KG, KP, KR, KZ, LC,	LK, LR, LS, LT,			
		LU, LV,	MA, MD,	MG, MK, MN,	MW, MX, MZ, NO, NZ,	PL, PT, RO, RU,			
		SD, SE,	SG, SI,	SK, SL, TJ,	TM, TR, TT, TZ, UA,	UG, US, UZ, VN,			
		YU, ZA,	ZW, AM,	AZ, BY, KG,	KZ, MD, RU, TJ, TM				
	RW:	GH, GM,	KE, LS,	MW, MZ, SD,	SL, SZ, TZ, UG, ZW,	AT, BE, CH, CY,			
		DE, DK,	ES, FI,	FR, GB, GR,	IE, IT, LU, MC, NL,	PT, SE, BF, BJ,			
		CF, CG,	CI, CM,	GA, GN, GW,	ML, MR, NE, SN, TD,	TG			
	CA 2385	484	AA	20010329	20000921				
	EP 1218	488	A1	20020703	EP 2000-963696	20000921			
	R:	AT, BE,	CH, DE,	DK, ES, FR,	GB, GR, IT, LI, LU,	NL, SE, MC, PT,			
				FI, RO, MK,					
	US 2003				US 2002-109812	20020329			

=> d 13 pi

- L6 ANSWER 13 OF 14 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
- SO Plant Journal, (July, 2001) Vol. 27, No. 2, pp. 171-178. print. ISSN: 0960-7412.

=> d 14 ab

- L6 ANSWER 14 OF 14 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 4
- The cDNA for the chloroplast-located homolog of bacterial RecA AB protein, designated recA-AT, was placed in a plasmid appropriate for in vitro transcription and translation. Translation with 35S-labeled Met permitted demonstration of uptake of the protein product into isolated pea chloroplasts, and processing to a mature size. Preliminary evidence for the first amino acid was estimated from results using both 35S-Met and 3H-Leu for in vitro transcription and translation, followed by uptake into chloroplasts and processing. The labeled protein was subject to sequential amino acid hydrolyses, and radioactivity was measured in each round. Induction of gene transcription in leaves infiltrated with the DNA-damaging agent, methyl methanesulfonate was shown by Northern blot analysis. Further constructs were made for over-expression of the gene in E. coli; and one out of many tried permitted production of some soluble protein. Extracts from transformed bacteria were shown to have RecA activity using the "POM" assay [Bertrand et al. (1993) Nucl. Acids Res. 21: 3653] for DNA strand transfer. The protein was purified to close to homogeneity using methods developed for E. coli RecA isolation.

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=> s ((maliga, p?) or (maliga p?))/au
L7 432 ((MALIGA, P?) OR (MALIGA P?))/AU
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=> s 17 and (chloroplast or plastid) L8 266 L7 AND (CHLOROPLAST OR PLASTID)

=> d 18 and (site specific or recombinase)

'AND' IS NOT A VALID FORMAT

'(SITE' IS NOT A VALID FORMAT

'SPECIFIC' IS NOT A VALID FORMAT

'OR' IS NOT A VALID FORMAT

'RECOMBINASE)' IS NOT A VALID FORMAT

In a multifile environment, a format can only be used if it is valid in at least one of the files. Refer to file specific help messages or the STNGUIDE file for information on formats available in individual files.

REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):ti

- L8 ANSWER 1 OF 266 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2005) on STN
- TI Affinity purification of the tobacco **plastid** RNA polymerase and in vitro reconstitution of the holoenzyme.

=> dup rem 19
PROCESSING COMPLETED FOR L9
L10 12 DUP REM L9 (13 DUPLICATES REMOVED)

NO VALID FORMATS ENTERED FOR FILE 'CAPLUS'
In a multifile environment, each file must have at least one valid format requested. Refer to file specific help messages or the STNGUIDE file for information on formats available in individual files.

REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT): ti

- L10 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Removal of heterologous sequences, such as selectable marker genes, from plastid genome by transiently expressed **site-specific** recombinases in higher plants
- L10 ANSWER 2 OF 12 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 1
- TI A novel approach to plastid transformation utilizes the phiC31 phage integrase.
- L10 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Plastid transformation in higher plants
- L10 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
- TI High level expression of immunogenic proteins in the plastids of higher plants and use thereof
- L10 ANSWER 5 OF 12 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 2
- TI Identification of functional lox sites in the plastid genome.
- L10 ANSWER 6 OF 12 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 3.
- TI The plastid clpP1 protease gene is essential for plant development.
- L10 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Use of integrases to promote the insertion of foreign DNA into the plastid genome
- L10 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 4
- TI Engineering the plastid genome of higher plants
- L10 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
- TI **Site-specific** recombination system to manipulate the plastid genome of higher plants
- L10 ANSWER 10 OF 12 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 5
- TI Efficient elimination of selectable marker genes from the plastid genome by the CRE-lox **site-specific** recombination system.
- L10 ANSWER 11 OF 12 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 6
- TI Site-specific factor involved in the editing of the psbL mRNA in tobacco plastids.
- L10 ANSWER 12 OF 12 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 7
- TI Introduction of a heterologous editing site into the tobacco plastid

genome: the lack of RNA editing leads to a mutant phenotype.

## => d pi

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ANSWER 1 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
L10
    PATENT NO.
               KIND
                               DATE
                                    APPLICATION NO.
                                                                DATE
                                          WO 2004-US6492
PΙ
    WO 2004078935
                        A2
                               20040916
                                                                 20040303
        W: AE, AE, AG, AL, AL, AM, AM, AT, AT, AU, AZ, AZ, BA, BB, BG,
            BG, BR, BR, BW, BY, BY, BZ, BZ, CA, CH, CN, CN, CO, CO, CR, CR,
            CU, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EC, EE, EE, EG, ES,
            ES, FI, FI, GB, GD, GE, GE, GH, GM, HR, HR, HU, HU, ID, IL, IN,
            IS, JP, JP, KE, KE, KG, KG, KP, KP, KR, KR, KZ, KZ, KZ, LC,
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            MZ, MZ, NA, NI
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## => d 3 so

- L10 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN SO Annual Review of Plant Biology (2004), 55, 289-313, 3 plates C1-C3 CODEN: ARPBDW
- => d 3 ab
- L10 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN A review. Plastids of higher plants are semi-autonomous organelles with a small, highly polyploid genome and their own transcription-translation machinery. This review provides an overview of the technol. for the genetic modification of the plastid genome including: vectors, marker genes and gene design, the use of gene knockouts and over-expression to probe plastid function and the application of sitespecific recombinases for excision of target DNA. Examples for applications in basic science include the study of plastid gene transcription, mRNA editing, photosynthesis and evolution. Examples for biotechnol. applications are incorporation of transgenes in the plastid genome for containment and high-level expression of recombinant proteins for pharmaceutical and industrial applications. Plastid transformation is routine only in tobacco. Progress in implementing the technol. in other crops is discussed.

## => d 8 ab

L10ANSWER 8 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 4 A review with refs. The plastid genome of higher plants is an attractive target for engineering because it provides readily obtainable high protein levels, the feasibility of expressing multiple proteins from polycistronic mRNAs and gene containment through the lack of pollen transmission. A chloroplast-based expression system that is suitable for the com. production of recombinant proteins in tobacco leaves has been developed recently. This expression system includes vectors, expression cassettes and site-specific recombinases for the selective elimination of marker genes. Progress in expressing proteins that are biomedically relevant, in engineering metabolic pathways, and in manipulating photosynthesis and agronomic traits is discussed, as are the problems of implementing the technol. in crops. Tools for engineering the plastid genome have reached the level of sophistication that would support the com. production of recombinant proteins in tobacco leaves.

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L10 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 4
     Current Opinion in Plant Biology (2002), 5(2), 164-172
     CODEN: COPBFZ; ISSN: 1369-5266
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L10 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
SO
     PCT Int. Appl., 83 pp.
     CODEN: PIXXD2
=> d 9 pi
    ANSWER 9 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
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In a multifile environment, a format can only be used if it is valid
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                                                     · DUPLICATE 5
     The Plant journal: for cell and molecular biology, July 2001. Vol. 27,
SO
     No. 2. p. 171-178
     Publisher: Oxford: Blackwell Sciences Ltd.
     ISSN: 0960-7412
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- L13 ANSWER 1 OF 9 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 1
- TI A novel approach to **plastid** transformation utilizes the phiC31 phage integrase.
- L13 ANSWER 2 OF 9 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 2
- TI Identification of functional lox sites in the plastid genome.
- L13 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Use of integrases to promote the insertion of foreign DNA into the plastid genome
- L13 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Site-specific recombination system to manipulate the plastid genome of higher plants
- L13 ANSWER 5 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN Chloroplasts for the production of recombinant proteins.
- L13 ANSWER 6 OF 9 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 3
- TI Efficient elimination of selectable marker genes from the plastid genome by the CRE-lox site-specific recombination system.
- L13 ANSWER 7 OF 9 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

  (2005) on STN DUPLICATE 4
- TI Conservation of RNA editing between rice and maize plastids: are most editing events dispensable?
- L13 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Reduction of the plastoquinone pool by exogenous NADH and NADPH in higher plant chloroplasts. Characterization of a NAD(P)H-plastoquinone oxidoreductase activity
- L13 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
- TI Evidence for a migration of ndh genes from the chloroplast to the nucleus in black pine

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L13 ANSWER 5 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN SO Photosynthesis Research, (2001) Vol. 69, No. 1-3, pp. 267. print. Meeting Info.: 12th International Congress on Photosynthesis. Brisbane, Australia. August 18-23, 2001. International Society of Photosynthesis Research.

CODEN: PHRSDI. ISSN: 0166-8595.